

# A Preliminary Study of the Effects of Nematocyst Removal on Agonistic Behavior in *Hermisenda*

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(1 Text figure)

## INTRODUCTION

ONE OF THE MOST CONSPICUOUS features of the opisthobranch *Hermisenda crassicornis* (Eschscholtz, 1831) is the many colorful cerata on the dorsal surface. These cerata are used in respiration (HYMAN, 1967) and also contain an extension of the digestive gland [in fact, the major portion of digestion apparently occurs in the cerata (BÜRGIN, 1965)]. The color of the cerata is determined partly by genetic factors and partly by the food eaten, as the color of the digestive gland will be a function of the type of food ingested (BÜRGIN, *op. cit.*). At the end of each ceras is a cnidosac. The nematocysts contained in the cnidosac are not produced by the *Hermisenda* but rather are contained in their chief food, hydroids. In some manner, the *Hermisenda* are able to prevent the hydroid's nematocysts from discharging when they are ingested. The nematocysts then pass through the digestive gland and eventually are stored in the cnidosac (EDMUNDS, 1966; NICOL, 1967).

The cerata, and especially the nematocysts, are thought to be of importance in interspecific encounters (EDMUNDS, 1966; THOMPSON & BENNETT, 1969). The cerata are easily autotomized and wriggle about violently after autotomization (MACFARLAND, 1966; personal observations). The cerata of most nudibranchs also contain mucus- and acid-secreting glands which are probably important in defense against predators (EDMUNDS, *op. cit.*). In fact, it is not certain whether the nematocysts or one of the secretions from the cerata are the most important in defense. It may be that the nematocysts are especially effective against fish where they would be released against the sen-

sitive tissues of the mouth, while acid secretions might be more effective against other predators (EDMUNDS, *op. cit.*). *Navanax*, an opisthobranch found in Southern California, is apparently unaffected by the nematocysts of *Hermisenda*, but rejects nudibranchs with a ceratal secretion of a higher acid content (PAINE, 1963).

Primarily on the basis of his histological investigations, STREBLE (1968) deduced that nudibranchs, specifically *Aeolidia papillosa* (Linnaeus, 1761), do not utilize their nematocysts as a form of defense. However, during previous investigations (ZACK, 1973, 1974a, 1974b) of intraspecific agonistic behavior in *Hermisenda* I have often seen them elongate and shake the cerata during encounters. And occasionally I have seen animals that are touched at one point on the body extend and point the cerata in the direction of the disturbance. Therefore, I have investigated the effects of nematocyst removal on the agonistic behavior of pairs of *Hermisenda*.

## MATERIALS AND METHODS

The *Hermisenda* used in this study were collected in tide pools on the central Oregon coast. The animals were maintained in large groups in sea water aquaria of 72 liter capacity with both an undergravel filter and an outside charcoal filter. Natural sea water was used throughout. The water temperature was maintained by cooling of the experimental room. The bulk of the observations was recorded from animals maintained on a daily cycle of 12 hours of light and 12 hours of dark. Illumination during the light period was provided by 2 60-watt incandescent light bulbs (approximately 200 lux), while illumination during the dark portion of the cycle was provided by a single 15-watt red incandescent bulb (less than 10 lux). The light period commenced at 0900 local time. The *Her-*

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*missenda* were fed a diet of fresh clam on which they thrived.

Four weeks prior to the initiation of behavioral observations almost all the cerata from 6 animals were removed with forceps. These animals were maintained in an aquarium of 7.21 capacity with continuous access to fresh clam. During this period the cerata regenerated. As the food supply did not consist of hydroids, from which the *Hermis-senda* ordinarily obtain nematocysts, the regenerated cerata were assumed to be virtually devoid of nematocysts (I had intended to confirm the absence of nematocysts histologically, but unfortunately before this could be accomplished the animals died).

For behavioral observation a pair of animals was "randomly" removed from the 71 home tank and placed in a standard finger bowl (10 cm diameter). A 25-watt incandescent light bulb was placed 40 cm above the finger-bowl for additional illumination. Recording usually began within one minute after the animals were placed in the observation bowl. I observed the animals for one hour from 2 to 6 hours after light onset. At the conclusion of the observation period the pair of *Hermis-senda* was replaced in the 71 home tank.

Recording was accomplished by verbal report with the aid of a standard monaural tape recorder. When it appeared that the 2 *Hermis-senda* were about to make contact the tape recorder was turned on and the time and position of the animals noted. Once contact was made the behavior pattern of each animal was recorded every  $1\frac{1}{2}$  seconds, paced by a metronome beating 40 times per minute. Following an observation period, the data were transcribed and later punched onto computer cards for detailed analysis. The computer analysis was performed using the Statistical Package for the Social Sciences (NIE *et al.*, 1970). All statistical tests used non-parametric techniques (SIEGEL, 1956).

For further information on the methods used and a complete lexicon of the behavior patterns recorded see ZACK, 1973, 1974a.

## RESULTS AND DISCUSSION

After the cerata had regenerated, the acystic *Hermis-senda* appeared healthy and normal behaving. Of the 76 encounters recorded in the 6 hours of observation, 77.6% of them (59 encounters) were non-agonistic. This is very similar to the proportion of non-agonistic encounters among control *Hermis-senda* (83.6%; ZACK, 1973, 1974a). No significant differences were found between acystic and control *Hermis-senda* in terms of the mean number of

encounters per observation period or in the duration of the encounters.

What about the structure of the encounters, that is, the organization of the behavior patterns? Table 1 is a matrix

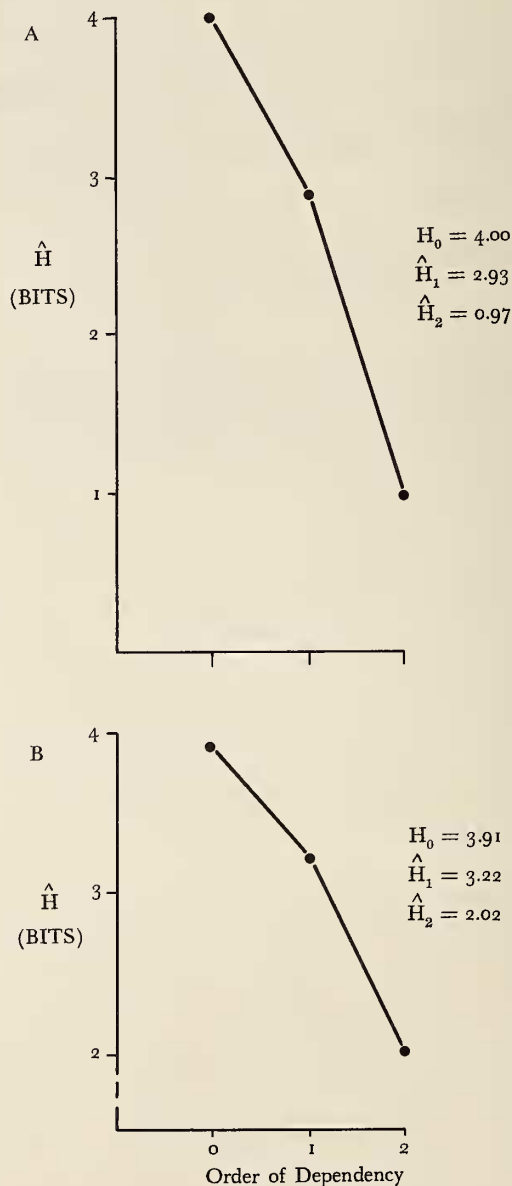


Figure 1

Amount of uncertainty associated with different orders of dependency for encounters of acystic *Hermis-senda*

(A) - with repeated behavior patterns

(B) - without repeated behavior patterns

Table 1

Transition matrix of preceding and following behavior patterns for the encounters occurring among acystic *Hermisenda*.  
 The upper number in each cell represents the actual number of occurrences of a particular combination, while the lower number is the percentage of times that the preceding pattern is followed by a particular pattern.  
 The lower numbers in the column headed Total represent the percentage for that column.

| Preceding Behavior Pattern | Following Behavior Pattern |           |            |           |             |            |             |            |          |        |             |          |            |            |            |            | Total       |
|----------------------------|----------------------------|-----------|------------|-----------|-------------|------------|-------------|------------|----------|--------|-------------|----------|------------|------------|------------|------------|-------------|
|                            | F                          | R         | L          | B         | S           | H          | E           | M          | X        | C      | K           | V        | T          | G          | D          | End        |             |
| Flagellate (F)             | 680<br>99.1                | 0<br>0    | 0<br>0     | 0<br>0    | 6<br>0.9    | 0<br>0     | 0<br>0      | 0<br>0     | 0<br>0   | 0<br>0 | 0<br>0      | 0<br>0   | 0<br>0     | 0<br>0     | 0<br>0     | 0<br>0     | 685<br>31.2 |
| Rear (R)                   | 0<br>0                     | 0<br>0    | 8<br>72.7  | 0<br>0    | 0<br>0      | 0<br>0     | 1<br>9.1    | 0<br>0     | 0<br>0   | 0<br>0 | 1<br>9.1    | 0<br>0   | 0<br>0     | 1<br>9.1   | 0<br>0     | 0<br>0     | 11<br>0.5   |
| Lunge (L)                  | 0<br>0                     | 1<br>3.4  | 10<br>34.5 | 0<br>0    | 0<br>0      | 0<br>0     | 1<br>3.4    | 4<br>13.8  | 0<br>0   | 0<br>0 | 3<br>10.3   | 0<br>0   | 0<br>0     | 2<br>6.9   | 1<br>3.4   | 7<br>24.1  | 29<br>1.3   |
| Bite (B)                   | 0<br>0                     | 2<br>20.0 | 0<br>0     | 5<br>50.0 | 0<br>0      | 0<br>0     | 1<br>10.0   | 0<br>0     | 0<br>0   | 0<br>0 | 1<br>10.0   | 0<br>0   | 0<br>0     | 0<br>0     | 0<br>0     | 1<br>10.0  | 10<br>0.5   |
| Sidle (S)                  | 0<br>0                     | 0<br>0    | 0<br>0     | 4<br>0.8  | 466<br>98.7 | 0<br>0     | 0<br>0      | 0<br>0     | 2<br>0.4 | 0<br>0 | 0<br>0      | 0<br>0   | 0<br>0     | 0<br>0     | 0<br>0     | 0<br>0     | 472<br>21.5 |
| Recoil (H)                 | 0<br>0                     | 1<br>1.3  | 4<br>5.0   | 0<br>0    | 0<br>0      | 5<br>6.3   | 31<br>38.8  | 1<br>1.3   | 0<br>0   | 0<br>0 | 22<br>27.5  | 0<br>0   | 3<br>3.8   | 1<br>1.3   | 8<br>10.0  | 4<br>5.0   | 80<br>3.6   |
| Withdraw (E)               | 0<br>0                     | 0<br>0    | 0<br>0     | 0<br>0    | 0<br>0      | 1<br>0.3   | 187<br>64.5 | 1<br>0.3   | 5<br>1.7 | 0<br>0 | 6<br>2.1    | 2<br>0.7 | 3<br>1.0   | 1<br>0.3   | 1<br>0.3   | 81<br>27.9 | 288<br>13.1 |
| Advance (M)                | 0<br>0                     | 4<br>3.3  | 2<br>1.6   | 0<br>0    | 0<br>0      | 5<br>4.1   | 4<br>3.3    | 46<br>37.7 | 1<br>0.8 | 0<br>0 | 6<br>4.9    | 0<br>0   | 21<br>17.2 | 16<br>13.1 | 6<br>4.9   | 11<br>9.0  | 122<br>5.6  |
| Twist (X)                  | 0<br>0                     | 0<br>0    | 0<br>0     | 0<br>0    | 0<br>0      | 0<br>0     | 5<br>62.5   | 0<br>0     | 0<br>0   | 0<br>0 | 0<br>0      | 0<br>0   | 0<br>0     | 0<br>0     | 0<br>0     | 3<br>37.5  | 8<br>0.4    |
| Move cerata (C)            | 0<br>0                     | 0<br>0    | 0<br>0     | 0<br>0    | 0<br>0      | 0<br>0     | 0<br>0      | 0<br>0     | 0<br>0   | 0<br>0 | 0<br>0      | 0<br>0   | 0<br>0     | 0<br>0     | 0<br>0     | 0<br>0     | 0<br>0      |
| Inactive (K)               | 0<br>0                     | 0<br>0    | 0<br>0     | 0<br>0    | 0<br>0      | 2<br>0.9   | 18<br>8.0   | 6<br>2.7   | 0<br>0   | 0<br>0 | 122<br>54.0 | 0<br>0   | 36<br>15.9 | 3<br>1.3   | 4<br>1.8   | 35<br>15.5 | 226<br>10.3 |
| Veer (V)                   | 0<br>0                     | 0<br>0    | 0<br>0     | 0<br>0    | 0<br>0      | 0<br>0     | 1<br>50.0   | 0<br>0     | 0<br>0   | 0<br>0 | 1<br>50.0   | 0<br>0   | 0<br>0     | 0<br>0     | 0<br>0     | 0<br>0     | 2<br>0.1    |
| Touch tentacle (T)         | 6<br>3.9                   | 1<br>0.6  | 2<br>1.3   | 0<br>0    | 0<br>0      | 45<br>29.2 | 6<br>2.7    | 44<br>28.6 | 0<br>0   | 0<br>0 | 37<br>24.0  | 0<br>0   | 10<br>6.5  | 0<br>0     | 2<br>1.3   | 0<br>0     | 153<br>7.0  |
| Touch foot (G)             | 0<br>0                     | 0<br>0    | 2<br>3.5   | 0<br>0    | 0<br>0      | 9<br>15.8  | 4<br>7.0    | 12<br>21.1 | 0<br>0   | 0<br>0 | 6<br>10.5   | 0<br>0   | 2<br>3.5   | 13<br>22.8 | 2<br>3.5   | 7<br>12.3  | 57<br>2.6   |
| Touch cerata (D)           | 0<br>0                     | 2<br>3.7  | 1<br>1.9   | 1<br>1.9  | 0<br>0      | 13<br>24.1 | 4<br>7.4    | 8<br>14.8  | 0<br>0   | 0<br>0 | 8<br>14.8   | 0<br>0   | 0<br>0     | 1<br>1.9   | 13<br>24.1 | 3<br>5.6   | 54<br>2.5   |
| Total                      | 686<br>31.2                | 11<br>0.5 | 29<br>1.3  | 10<br>0.5 | 472<br>21.5 | 80<br>3.6  | 263<br>12.0 | 122<br>5.6 | 8<br>0.4 | 0<br>0 | 213<br>9.7  | 2<br>0.1 | 75<br>3.4  | 38<br>1.7  | 37<br>1.7  | 152<br>6.9 | 2198        |

showing the behavior pattern transitions occurring among the acystic *Hermisenda*. It is obvious that there is some dependence between preceding and following patterns. The amount of uncertainty (H) at different orders of dependency (Figure 1) gives one some insight into the structure of the encounters (ATTNEAVE, 1959; CHATFIELD & LEMON, 1970). The large decrease in uncertainty from H<sub>1</sub> to H<sub>2</sub>, called T<sub>2</sub>, combined with the low value of

H<sub>2</sub> strongly implies a second order dependency<sup>2</sup>. This is similar to what was found with the control animals, except with the acystic animals it is much more marked. Calculation of the uncertainty after removal of the repeated behavior patterns (the diagonal of Table 1) reduces T<sub>1</sub> (H<sub>0</sub>

<sup>2</sup> All values of H<sub>1</sub> and T<sub>1</sub>, except H<sub>0</sub>, are estimates calculated from the sample data of the respective population parameters

Table 2

Comparison of the sequence of behavior patterns. The capital letters represent the following behavior pattern for each specific preceding pattern which has the largest ratio of observed to expected occurrences. The lower case letters represent the second largest ratio. In some cases there was only one or no following behavior patterns and therefore certain letters are missing. A(a) = control animals. B(b) = acystic animals.

| Preceding Behavior Pattern | Following Behavior Pattern |        |        |        |        |   |        |        |        |   |   |   |        |        |   |     |
|----------------------------|----------------------------|--------|--------|--------|--------|---|--------|--------|--------|---|---|---|--------|--------|---|-----|
|                            | F                          | R      | L      | B      | S      | H | E      | M      | X      | C | K | V | T      | G      | D | End |
| Flagellate (F)             |                            |        |        |        | A<br>B | a |        |        |        |   |   |   |        |        |   |     |
| Rear (R)                   |                            |        | A<br>B | a      |        |   |        |        |        |   |   |   |        |        |   | b   |
| Lunge (L)                  |                            | a<br>B |        | A      |        |   |        |        |        |   |   |   |        |        |   | b   |
| Bite (B)                   |                            | B      | a      |        |        |   | b      |        |        | A |   |   |        |        |   |     |
| Sidle (S)                  |                            |        |        | a<br>B |        |   |        |        | b      | A |   |   |        |        |   |     |
| Recoil (H)                 | a                          |        |        |        |        |   |        |        |        |   |   | A |        |        |   |     |
| Withdraw (E)               |                            |        |        |        |        |   | B      |        | A<br>b |   |   | B |        |        | b | a   |
| Advance (M)                |                            | a<br>b |        |        |        |   |        |        |        |   |   |   |        | A<br>B |   |     |
| Twist (X)                  |                            |        |        |        |        |   | A<br>B |        |        |   |   |   |        | a      |   |     |
| Move cerata (C)            |                            |        |        | A      |        |   | a      |        |        |   |   |   |        |        |   | b   |
| Inactive (K)               |                            |        |        |        |        |   |        |        |        |   |   |   | a<br>B |        | A | b   |
| Veer (V)                   |                            |        |        |        | a      |   | A<br>B |        |        |   | b |   |        |        |   |     |
| Touch tentacle (T)         | a<br>B                     |        |        |        |        | A | b      |        |        |   |   |   |        |        |   |     |
| Touch foot (G)             |                            | a      |        |        |        | b |        | A<br>B |        |   |   |   |        |        |   |     |
| Touch cerata (D)           |                            | b      |        | B      |        | A |        | a      |        |   |   |   |        |        |   |     |

minus  $H_1$ ) and  $T_2$  to levels quite similar to that found in control animals (Figure 1B). This result indicates that one difference between control and acystic encounters is the amount of repeated behavior patterns (probably a good indicator of bout length). In fact, repeated behavior patterns constitute 70.8% of the patterns of acystic *Hermisenda* and only 55.6% of the patterns among control animals. Examination of the distribution of repeated behavior patterns indicates that the acystic animals are much more likely to repeat flagellation and sidling than the control animals. It should be pointed out that flagellation and sidling only occur in 2 encounters and it may be that the very long duration of these encounters biased the data.

Having determined that the structure of the encounters (except possibly for the repeated behavior patterns) is very similar in acystic and control *Hermisenda*, I next examined the sequence of the patterns themselves. This was done after removal of the repeated patterns, as one is most interested in the changes from one pattern to another. Table 2 demonstrates the similarity of the behavior sequences of control and acystic animals. The acystic animals differ from the control animals only in the behavior patterns following biting, recoiling, moving the cerata, and touching the cerata. Following biting the controls usually move the cerata while the acystics rear. After a recoil the controls usually veer and the acystics usually

withdraw. I never recorded moving of the cerata among the acystic animals. This seems to indicate that either some permanent damage was done by removal of the cerata, or complete regeneration had not occurred. Most interesting is the fact that following touching of the cerata the control animals usually recoil, while the acystic animals usually bite. It thus appears that removal of the nematocysts changes this one behavior pattern sequence from the normal defensive mode (recoiling) to an offensive mode (biting). Therefore, the nematocysts do have a small, but definite, influence on intraspecific interactions.

Winners of encounters show greatly increased amounts of rearing, lunging, biting, advancing, and touching the foot or cerata. Losers show increased amounts of withdrawal and twisting. These differences between winners and losers are the same as was seen for control animals (ZACK, 1974a).

In conclusion, it appears that the removal of the nematocysts, while modifying some of the more subtle aspects, such as the most likely behavior pattern following touching of the cerata, does not have a large or dramatic effect on the intraspecific agonistic behavior of *Hermisenda*.

There are limitations to the present study. (1) I do not know whether there were any nematocysts remaining in the regenerating cerata. It seems unlikely that very many could remain, especially in light of the study by KEPNER (1943). He found that in the nudibranch *Aeolis pilata* Gould, 1870, within 4 hours of ingesting food containing nematocysts, all the nematocysts were either digested or in the cnidosacs of the cerata. Since *Hermisenda* used in this study had been on a diet free of nematocysts for 2 to 5 days prior to the removal of the cerata it is unlikely that any remained in the gut to resupply the regenerated cerata. However, since comparable studies have not been performed on *Hermisenda* and I was unable to examine the animals used in this study for the presence of nematocysts, I cannot be certain that all the nematocysts were removed. (2) I also cannot differentiate between the effects of the operation *per se* and the removal of the nematocysts. This would require a group of animals which had all the cerata removed and were then maintained on a normal diet of hydroids with nematocysts. (3) Another complication is that the acystic *Hermisenda* lived in a small tank for approximately one month prior to being observed. Although the amount of free surface area per animal on which they could locomote was not too different from that in the main tank, the small size of the tank itself may have altered their normal responses. Obviously, another control group – of isolated,

non-manipulated – animals would be useful. (4) Another possible difficulty is that the group of animals from which the acystic animals were drawn is itself different from the animals used as controls. This is unlikely to be a major factor as the group of animals from which the acystic animals were derived accounts for more than 60% of the encounters (and observation periods) of the control group.

The consequences of the last 3 points would all lead to differences in the behavior of the acystic animals and the control animals. As no striking differences were found, it is highly unlikely that any of them are of great importance.

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